

## Porcine and recombinant zona pellucida vaccines as immunocontraceptives for donkeys in the Caribbean

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Immunocontraception has been investigated as an alternative to hormone manipulation and lethal methods of fertility control in wildlife for almost 30 years. Porcine zona pellucida (PZP) vaccine has been shown to utilize an animal's immune system to prevent pregnancies temporarily and reversibly in multiple species. This study investigated PZP vaccination as a method of practical population control in feral donkeys (*Equus asinus*) in the tropics. In addition, the study investigated a novel recombinant zona pellucida vaccine (recZP) as an alternative for fertility management in donkeys.

Twenty-five feral female donkeys of proven fertility were captured on Nevis and transported to Ross University School of Veterinary Medicine (RUSVM). All animal protocols were approved through RUSVM IACUC review. Jennies allocated to Group 1 (n=9) received the recZP vaccine in complete modified Freund's adjuvant (CMFA) and thereafter two booster vaccines in incomplete Freund's adjuvant (IFA) 5 weeks and in sterile saline 10 weeks later. Jennies allocated to Group 2 (n=8) received the PZP vaccine in CMFA and one booster in IFA 5 weeks later. Those allocated to Group 3 (n=8) received an initial injection of CMFA and second injection of IFA 5 weeks later and acted as controls. All treatments were administered intramuscularly by injection into the left and right gluteal muscles (1st and 2nd injections), and the left pectoral muscles (3rd injection in Group 1). Trans-rectal ultrasonography was performed weekly on each jenny to monitor ovarian and follicular development and cyclical reproductive activity. Five weeks after the final injection, one jack was placed with each group of jennies. Estrus detection, mounting behaviors, and breeding were recorded when observed. Weekly trans-rectal observations were continued to visualize the reproductive tracts and ovaries and to identify pregnancies. Jacks were rotated through the groups every three weeks.

All donkeys were cycling normally prior to injection with obvious estrus signs detected when in proximity to a jack. Sterile abscesses at injection sites were observed in 9/9 (100%) jennies in Group 1, 7/8 (87.5%) jennies in Group 2 and 3/8 (37.5%) jennies in Group 3. Three months after the final injection, 7/9 (78%) jennies in Group 1 and 6/8 (75%) jennies in Group 2 had no detectable follicular development nor estrus signs. No pregnancies were found in either of the treatment groups at twelve weeks post-treatment. In contrast, 6/8 (75%) of the control group were pregnant and all had shown follicular development.

Despite the injection site reactions, both PZP and recZP vaccines were shown to be effective as non-hormone based contraceptives with potential for application in fertility management programs in donkeys. Further investigation of these vaccines, using alternative adjuvants providing similar contraceptive efficacy without the associated adverse reactions, may benefit future population control programs for feral donkeys in the Caribbean.